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Comparison of the Mycobacteria Growth Indicator Tube with MB Redox, Löwenstein-Jensen, and Middlebrook 7H11 Media for Recovery of Mycobacteria in Clinical Specimens

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The rate of recovery and the mean time to detection of mycobacteria in clinical specimens were evaluated with two nonradiometric broth-based systems, the Mycobacteria Growth Indicator Tube (MGIT) and MB Redox systems. The data obtained for each system were compared with each other and with those obtained with the Löwenstein-Jensen (LJ) and Middlebrook 7H11 reference media. A total of 117 mycobacterial isolates (*Mycobacterium tuberculosis*, $n = 112$; nontuberculous mycobacteria, $n = 5$) were detected in 486 clinical specimens. The recovery rates for *M. tuberculosis* were 91 of 112 (81.3%) isolates with MGIT and 81 of 112 (72.3%) isolates with MB Redox. The combination of MGIT plus MB Redox recovered 104 of the 112 (92.9%) *M. tuberculosis* isolates. MGIT plus LJ plus Middlebrook 7H11 recovered 106 of the 112 (94.6%) isolates, MB Redox plus LJ plus Middlebrook 7H11 recovered 99 of the 112 (88.4%) isolates, and LJ plus Middlebrook 7H11 recovered 84 of the 112 (75.0%) isolates. The mean time to detection of *M. tuberculosis* in smear-positive specimens was 7.2 days with MGIT, 6.9 days with MB Redox, 20.4 days with LJ, and 17.6 days with Middlebrook 7H11. The mean time to detection of *M. tuberculosis* in smear-negative specimens was 19.1 days with MGIT, 15.5 days with MB Redox, 25.8 days with LJ, and 21.6 days with Middlebrook 7H11. The contamination rates were 4.4, 3.8, 2.1, and 2.7% for MGIT, MB Redox, LJ, and Middlebrook 7H11, respectively. In conclusion, MGIT and MB Redox can be viable tools in the routine mycobacteriology laboratory.

The rapid diagnosis of tuberculosis is important if the necessary control and prevention steps are to be taken in due time, the spread of the disease is to be limited, the administration of inadequate therapy is to be avoided, and the costs of hospitalization are to be reduced. Clinical and radiological findings permit only a presumptive diagnosis of tuberculosis. Conventional microscopy for acid-fast bacteria (AFB) is a rapid procedure but has a low sensitivity (4, 6). The application of nucleic acid amplification methods in mycobacteriology promised radical changes. Unfortunately, the routine diagnostic use of these procedures had a number of major drawbacks, in particular, the high variation in sensitivity, the detection of nonviable bacteria, and the high costs of these tests (7, 8). A definitive diagnosis of tuberculosis is still dependent on the isolation of *Mycobacterium tuberculosis* by cultivation. However, cultivation on solid media, such as that of Löwenstein-Jensen (LJ), is both time-consuming, taking up to 6 to 8 weeks, and insensitive (4, 6). Introduction of the liquid medium-based BACTEC 460 TB radiometric system (Becton-Dickinson Diagnostic Instrument Systems, Sparks, Md.) has led to a considerable shortening of the time required for the detection of mycobacteria and has increased the sensitivity of isolation (1, 2). The main limitations of the system are the high cost of disposal of the radioactive waste and the need for instrumentation.

Two nonradioactive broth-based culture methods were recently introduced. These are known commercially as the Mycobacteria Growth Indicator Tube (MGIT; BBL Becton Dickinson Microbiology Systems, Cockeysville, Md.) and the MB Redox (Biotest AG, Dreieich, Germany). MGIT contains 4 ml of modified Middlebrook 7H9 broth with an oxygen quenching-based fluorescent sensor. The large amount of oxygen initially dissolved in the broth quenches the fluorescence, but the growth of any microorganisms that might be present, such as mycobacteria, is accompanied by the consumption of the oxygen, which allows the indicator to fluoresce under 365-nm UV light. MB Redox uses 4 ml of modified, serum-supplemented Kirchner medium with a colorless tetrazolium salt as a growth indicator. During bacterial growth, the tetrazolium salt is reduced to a pink-, red-, or violet-colored formazan, and the presence of one of these colors indicates the presence of mycobacteria. The formazan is water insoluble and is secreted to the cell surface in a granular form. Furthermore, MB Redox also contains a special vitamin complex which provides for a considerable acceleration of the growth of mycobacteria compared with the time for growth to appear with standard Kirchner medium and promotes the formation of formazan.

In previous studies, MGIT has been reported to have a sensitivity, a rapidity, and recovery rates comparable to those of BACTEC 460 TB (3, 9). MB Redox has also been found to be as effective as BACTEC 460 TB, but to our knowledge only one report on its effectiveness is available (5). The aim of the present study was to compare these two recently introduced media, MGIT and MB Redox, with each other and with the reference LJ and Middlebrook 7H11 media in terms of recovery rates, the mean times required to detect mycobacteria in clinical specimens, and the contamination rates.

MATERIALS AND METHODS

Specimens. A total of 486 clinical specimens received for routine mycobacterial cultivation were processed between 12 February 1997 and 4 March 1997. These were 405 sputum, 37 bronchoalveolar lavage or bronchial mucus aspirate, 24 gastric juice, 18 urine, and 2 stool specimens.

Specimen processing. All clinical specimens were digested and decontaminated by the *N*-acetyl-L-cysteine–NaOH method, as described by Kent and Ku-

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TABLE 1. Rates of recovery of mycobacteria and contaminants with MGIT, MB Redox, LJ, and Middlebrook 7H11

Medium	No. (%) of isolates recovered[a]			
	All isolates (n = 117)	MTB (n = 112)	NTM (n = 5)	Contaminants (n = 24)
MGIT	93 (79.5)	91 (81.3)	2 (40)	21 (4.3)
MB Redox	84 (71.8)	81 (72.3)	3 (60)	19 (3.9)
LJ	73 (62.4)	72 (64.3)	1 (20)	10 (2.1)
Middlebrook 7H11	68 (58.1)	68 (60.7)	0	13 (2.7)

[a] χ^2 test for differences in recovery of mycobacteria and *M. tuberculosis*: MGIT versus solid media, $P < 0.01$ (significant); MB Redox versus Middlebrook 7H11, $P < 0.05$ (significant). χ^2 test for differences in contamination: no significant differences. MTB, *M. tuberculosis*; NTM, nontuberculous mycobacteria.

bica (4), with a final NaOH concentration of 1%. After decontamination, smears were prepared from the concentrated sediments of the specimens for Ziehl-Neelsen (ZN) acid-fast staining (4).

Preparation of MGIT and MB Redox for inoculation. Prior to inoculation, 0.5 ml of oleic acid-albumin-dextrose-catalase (OADC; BBL catalog no. 4345116) and 0.1 ml of a mixture containing polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin (PANTA; BBL catalog no. 4345114) were added to each MGIT vial (BBL catalog no. 4345111).

MB Redox (Biotest catalog no. 913300) is a ready-to-use medium that contains both OADC and the antibiotics polymyxin B, amphotericin B, carbenicillin, and trimethoprim (PACT).

Quality control. Quality control was performed on each new lot of MGIT or MB Redox, with tests performed with the reference strains *M. tuberculosis* ATCC 27294, *Mycobacterium kansasii* ATCC 12478, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus epidermidis* ATCC 12228. In accordance with the manufacturer's instructions, multiple serial mycobacterial and bacterial dilutions derived from a 0.5 McFarland nephelometer standard were made in sterile saline and were then inoculated into MGIT and MB Redox vials.

Inoculation and cultivation of clinical specimens. One-half milliliter of the processed specimen was inoculated into MGIT, 0.5 ml was inoculated into MB Redox, 0.2 ml was inoculated onto each of two LJ slants (prepared in our laboratory), and 0.2 ml was inoculated onto a Middlebrook 7H11 slant which contained PACT, in addition to OADC (Heipha Diagnostika, Dreieich, Germany). All inoculated media were incubated at 37°C. MGIT vials were examined daily with 365-nm UV light (using a Wood's lamp) for 8 weeks. Any vials that showed fluorescence comparable to that of a positive chemical control (a 0.4% sodium sulfite solution in an empty and uninoculated MGIT vial) and growth control (quality control vial with *M. tuberculosis* ATCC 27294) were considered positive. If no fluorescence was seen after 8 weeks, as for the negative control (uninoculated MGIT vial), the MGIT vial was regarded as negative.

MB Redox vials were also examined daily for 8 weeks. Examination was performed in two steps: first without shaking and then with slight shaking of the vials. MB Redox vials that exhibited pink, red, or violet particles comparable to those in a positive growth control (quality control vial with *M. tuberculosis* ATCC 27294) were considered positive. Vials without such a color, as for the negative control (uninoculated MB Redox vial), were considered to be negative.

LJ and Middlebrook 7H11 slants were examined weekly for 8 weeks for the visible appearance of colonies. After the confirmation of mycobacterial growth in a liquid or solid medium, the parallel solid media were read daily.

On the day of detection, all positive liquid or solid media were examined by ZN staining to confirm the presence of AFB, and the colonies were subcultured onto Columbia agar with 5% sheep blood (bioMerieux Microbiology Systems, Marcy l'Etoile, France) to check for contaminants. MGIT and MB Redox vials without fluorescence or color formation but with nonhomogeneous turbidity and small grains or flakes in the culture were also considered presumptively positive and were screened in the same way. Cultures found to be AFB positive by microscopy were subcultured onto two LJ slants for identification by means of conventional biochemical tests (4, 6). Contaminants were not identified.

Statistical analysis. The χ^2 test was used to evaluate differences between recovery and contamination rates in different media. Analysis of variance (ANOVA) and the Newman-Keuls test were used to establish significant differences in relation to the duration of growth by using the Primer for Biostatistics program (version 3.01 [1992]; McGraw-Hill, New York, N.Y.).

RESULTS

Cultures positive for AFB were obtained for a total of 117 (24.1%) specimens, of which 22 (18.8%) were direct smear positive and 95 (81.2%) were direct smear negative. The mycobacterial species identified were *M. tuberculosis* (n = 112), *Mycobacterium xenopi* (n = 3), and *M. kansasii* (n = 2).

In five MGIT vials without fluorescence, positivity was suggested by the observation of a nonhomogeneous turbidity and small grains, but AFB positivity was confirmed by ZN smears and subculture on LJ.

The recovery rates for MGIT, MB Redox, LJ, and Middlebrook 7H11 are presented in Table 1. The recovery rates obtained for *M. tuberculosis* were 91 of 112 (81.3%) isolates with MGIT, 81 of 112 (72.3%) isolates with MB Redox, 72 of 112 (64.3%) isolates with LJ, and 68 of 112 (60.7%) isolates with Middlebrook 7H11. Statistically significant differences were found between MGIT and LJ ($P < 0.01$) and between MGIT and Middlebrook 7H11 ($P < 0.01$). Twelve *M. tuberculosis* isolates grew only on MGIT, six isolates grew only on MB Redox, five isolates grew only on LJ, and two isolates grew only on Middlebrook 7H11.

MGIT detected all 22 smear-positive specimens, while the other three media each detected 21 of the 22 smear-positive specimens. All smear-positive specimens contained *M. tuberculosis*. For the smear-negative specimens, the *M. tuberculosis* recovery rates were 69 of 90 (76.6%) isolates with MGIT, 60 of 90 (66.6%) isolates with MB Redox, 51 of 90 (56.6%) isolates with LJ, and 47 of 90 (52.2%) isolates with Middlebrook 7H11. Statistically significant differences were found between MGIT and LJ ($P < 0.01$) and between MGIT and Middlebrook 7H11 ($P < 0.05$). The difference between MB Redox and Middlebrook 7H11 was close to being statistically significant ($P = 0.069$).

Recovery rates on media in the following different combinations were also compared: MGIT plus MB Redox (combination A), MGIT plus LJ plus Middlebrook 7H11 (combination B), MB Redox plus LJ plus Middlebrook 7H11 (combination C), and LJ plus Middlebrook 7H11 (combination D). Combination A recovered 104 of the 112 (92.9%) *M. tuberculosis* isolates, combination B recovered 106 of the 112 (94.6%) isolates, combination C recovered 99 of the 112 (88.4%) isolates, and combination D recovered 84 of the 112 (75.0%) isolates. Statistically significant differences were demonstrated between combinations A and D ($P < 0.01$), combinations B and D ($P < 0.01$), and combinations C and D ($P < 0.01$). Rates of recovery for all mycobacterial and *M. tuberculosis* isolates observed with the combinations listed above are listed in Table 2.

The mean times from inoculation to the detection of growth of mycobacteria and *M. tuberculosis* in the different media are detailed in Table 3. The mean (range) times to detection of *M. tuberculosis* were 16.5 (2 to 42), 13.3 (2 to 33), 24.2 (13 to 59), and 20.4 (7 to 53) days with MGIT, MB Redox, LJ, and Middlebrook 7H11, respectively. ANOVA and the Newman-Keuls test revealed statistically significant differences ($P < 0.001$ and $P < 0.05$, respectively) between MGIT, MB Redox, LJ, and Middlebrook 7H11. For smear-positive specimens, *M. tuberculosis* was detected after a mean (range) of 7.2 (2 to 10) days with MGIT, 6.9 (2 to 11) days with MB Redox, 20.4 (13 to 46) days with LJ, and 17.6 (7 to 27) days with Middle-

TABLE 2. Rates of recovery of mycobacteria and MTB with liquid and solid media in combination

Isolate (no. of isolates)	No. (%) of isolates recovered[a]			
	Combination A	Combination B	Combination C	Combination D
Mycobacteria (n = 117)	109 (93.2)	108 (92.3)	103 (88.0)	85 (72.6)
MTB (n = 112)	104 (92.9)	106 (94.6)	99 (88.4)	84 (75.0)

[a] χ^2 test for differences in recovery of mycobacteria and *M. tuberculosis*: combination A, B, or C versus combination D, $P < 0.01$ (significant). MTB, *M. tuberculosis*.

TABLE 3. Mean times to detection of mycobacteria and MTB in clinical specimens

Medium	Mean time to detection (range [days])[a]		
	Mycobacteria	Smear-positive MTB	Smear-negative MTB
MGIT	17.0 (2–56)	7.2 (2–10)	19.1 (14–42)
MB Redox	14.7 (2–58)	6.9 (2–11)	15.5 (12–33)
LJ	24.6 (13–59)	20.4 (13–46)	25.8 (16–59)
Middlebrook-7H11	20.4 (7–53)	17.6 (7–27)	21.6 (14–53)

[a] By ANOVA, $P < 0.001$. Newman-Keuls test for differences in mean times to detection of mycobacteria: MGIT versus LJ, $P < 0.05$ (significant); MB Redox versus LJ, $P < 0.05$ (significant). Newman-Keuls test for differences in mean times of detection of *M. tuberculosis*: MGIT versus MB Redox versus LJ versus Middlebrook 7H11, $P < 0.05$ (significant). MTB, *M. tuberculosis*.

brook 7H11. For smear-negative specimens, the mean (range) times to detection were 19.1 (14 to 42), 15.5 (12 to 33), 25.8 (16 to 59), and 21.6 (14 to 53) days with MGIT, MB Redox, LJ, and Middlebrook 7H11, respectively.

The low number of nontuberculous mycobacteria did not allow an exact statistical comparison for this group. Studies with a larger number of isolates are needed to determine the reliability of MGIT and MB Redox for the detection of nontuberculous mycobacteria. The contamination rates for MGIT, MB Redox, LJ (a specimen was classified as contaminated if both parallel slants displayed nonmycobacterial growth), and Middlebrook 7H11 were 21 of 486 (4.3%), 19 of 486 (3.9%), 10 of 486 (2.1%), and 13 of 486 (2.7%), respectively (Table 1). Statistical analysis did not reveal any significant difference.

DISCUSSION

After decades of decline, the incidence of tuberculosis has again been on the increase in Hungary in recent years, and there is a need for new, rapid, and effective laboratory cultivation methods. The recently introduced broth-based culture systems MGIT and MB Redox have been reported to satisfy these requirements (3, 5, 9, 10). Because those previous studies have found both MGIT and MB Redox to be comparable to the BACTEC 460 TB system, that system was not included in the present evaluation. The parallel inoculation into a BACTEC 12B vial was also limited by the restricted amount of sediment. A 0.5-ml increase in the volume of saline used for resuspension of the sediment after decontamination could have decreased the sensitivity due to specimen dilution.

As regards the rates of recovery of *M. tuberculosis* by each method, both liquid media were better than the solid media, in accord with other reports (3, 5, 9, 10). In our study, MGIT yielded the highest rate of recovery of *M. tuberculosis*. The differences between MGIT and the solid media were statistically significant, in contrast to the findings of Casal et al. (3) and Pfyffer et al. (9). However, while Pfyffer et al. (9) detected 70 (38.9%) smear-positive specimens and Casal et al. (3) detected 174 (82.1%) smear-positive specimens, in our study only 22 (18.8%) specimens were smear positive. This higher rate of smear-positive specimens may have reduced the difference in the rate of recovery between MGIT and the solid media. Thus, it did not reach a statistically significant level in their studies. Our results were also in contrast to the findings of Naumann et al. (5), because our statistical analysis of the rates of recovery of *M. tuberculosis* did not reveal significant differences between MB Redox and the solid media. However, the statistically significant difference between MB Redox and solid media observed by Naumann et al. (5) may be due to the lower number of isolates in their study (50 *M. tuberculosis* and 16 nontuberculous mycobacterial isolates in 974 specimens). Although there was no statistically significant difference between the two liquid media, our results indicate that the MGIT method may be more sensitive than the MB Redox method.

Whereas MB Redox, LJ, and Middlebrook 7H11 all failed to recover 1 of the 22 smear-positive isolates, MGIT detected all of them. For smear-negative *M. tuberculosis* isolates, we found the same statistical differences in recovery rates between the different media as described above for all *M. tuberculosis* isolates. However, the difference between the recovery rates with MB Redox and Middlebrook 7H11 for specimens that were smear negative for *M. tuberculosis* was close to being significant ($P = 0.069$).

None of the four media detected all of the mycobacterial isolates. The yield of mycobacterial isolates increased with the number of media used in combination, as observed by others (9, 10). MGIT detected 14 more mycobacterial isolates and MB Redox detected 9 more mycobacterial isolates when they were each used in combination with the two solid media. Our data indicate a need for the inclusion of a solid medium in the primary isolation procedure because six mycobacterial isolates grew only on LJ and two grew only on Middlebrook 7H11. These findings lend support to the recommendation that a solid medium should not be used alone but should be used in combination with a broth-based system as a "gold standard" for the optimum results (4, 6, 9).

The fact that no statistical difference was found between the two gold standards in terms of the rates of recovery of *M. tuberculosis* and the observation that these recovery rates compare well with those obtained with BACTEC 12B medium suggest that either combination could replace the standard consisting of BACTEC 12B medium plus solid medium (1, 9). Pfyffer et al. (9) recently demonstrated that a combination of two liquid media (MGIT and BACTEC 12B) was more efficient than combinations of liquid and solid media (9). They also recommended definition of the efficacy of combined liquid media which do not contain radioisotopes. In our study, the combination of MGIT and MB Redox (combination A) displayed a slightly lower rate of recovery of *M. tuberculosis*, without any statistically significant difference, than those for combinations B and C. Our findings do not support the assumption that the use of two nonradioactive liquid media is more efficient than the use of a liquid medium plus a solid medium. Statistically significant differences were found when combination A, B, or C was compared with the combination of the solid media (combination D), as observed from a comparison of MGIT, BACTEC 12B, and solid media by Pfyffer et al. (9).

The mean times to detection of *M. tuberculosis* from smear-positive specimens with MGIT and MB Redox were comparable (7.2 versus 6.9 days). These are shorter than those in previous reports for MGIT: 9.0 days by Casal et al. (3), 9.9 days by Pfyffer et al. (9), or 15.3 days by Rivera et al. (10). In our study, however, the number of smear-positive specimens was much lower. The mean times to detection of *M. tuberculosis* in smear-positive specimens were much shorter with MGIT and MB Redox than those with the solid media.

The mean time to detection of *M. tuberculosis* in smear-negative specimens was longer with MGIT than with MB Redox (19.1 versus 15.5 days). The time to detection with MGIT accorded well with the times of 20.3 days reported by Pfyffer et al. (9) and 18.6 days reported by Rivera et al. (10) but was inconsistent with the 14.0 days found by Casal et al. (3). The time to detection with MB Redox (15.5 days) was lower than those observed by Pfyffer et al. (9) and Rivera et al. (10) but was in line with that given by Casal et al. (3) with MGIT. We

have found no previously published data on the times of recovery of smear-positive and -negative specimens with MB Redox with which to compare our findings. However, our data indicate that the MB Redox method may be much faster than the MGIT method for the recovery of *M. tuberculosis* from smear-negative specimens.

An excellent mean time to recovery was obtained for smear-negative, *M. tuberculosis*-positive specimens on Middlebrook 7H11 slants (21.6 days), and this mean time to recovery was comparable to that with MGIT (19.1 days). Both liquid media were significantly faster than LJ for the detection of smear-negative, *M. tuberculosis*-positive specimens, and detection with MB Redox was also significantly faster than that with Middlebrook 7H11.

The MGIT and MB Redox systems are rapid, sensitive, and easy to handle, and they do not require additional costly instrumentation. The contamination rates of the two liquid media were also acceptable and did not cause any problems. Although the addition of OADC and PANTA to MGIT is an inconvenient extra step, the lack of the antibiotic mixture in the original broth allows a longer shelf-life. Furthermore, no refrigeration is needed during storage, unlike with the ready-to-use MB Redox with PACT. The PACT present in MB Redox may also decrease the mycobacterial growth in specimens from sterile body sites, while the inoculation of such specimens into MGIT without the addition of PANTA allows this problem to be avoided. In our experience, the examination of MB Redox for growth positivity is easier because the reading of MGIT specimens with UV light can be stressful to the eyes. Although the MGIT system is a bit more expensive than MB Redox, both media remain cost-effective in laboratories that cannot afford expensive instrumentation.

In conclusion, MGIT and MB Redox can be viable tools in the routine mycobacteriology laboratory. Further evaluations are needed to assess their efficiencies in comparison with that of the BACTEC 460 TB system and for the detection of nontuberculous mycobacteria.

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